PATENT
Docket No.: **19226/2201 (R-5771)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Szyperski et al.	)	Examiner:
			)	Yelena G. Gakh
Serial No.	:	10/628,818	)	
			)	Art Unit:
Filed	:	July 28, 2003	)	1743
			)	
For	:	PHASE SENSITIVELY-DETECTED REDUCED DIMENSIONALITY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY FOR RAPID CHEMICAL SHIFT ASSIGNMENT AND SECONDARY STRUCTURE DETERMINATION OF PROTEINS	)	

REQUEST FOR RECONSIDERATION

Mail Stop
Commissioner for Patents
P.O. Box 1450
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Dear Sir:

In response to the December 28, 2006, office action, reconsideration is respectfully requested.

The use of triple resonance (TR) nuclear magnetic resonance (NMR) experiments for the resonance assignment of polypeptide chains *via* heteronuclear scalar connectivities is a standard approach which neatly complements the assignment protocol based on ^{1}H–^{1}H nuclear Overhauser effects (NOE). In addition, triple resonance NMR spectra are highly amenable to a fast automated analysis, yielding the $^{13}C^{\alpha/\beta}$ chemical shifts at an early stage of the assignment procedure. This enables both the identification of regular secondary structure elements without reference to NOEs and the derivation of (ϕ,ψ)-angle constraints which serve to reduce the number of cycles consisting of nuclear Overhauser enhancement spectroscopy (NOESY) peak assignment and structure calculation.

NMR assignments are prerequisite for NMR-based structural biology and, thus, for high-throughput (HTP) structure determination in structural genomics and for exploring structure-activity relationships (SAR) by NMR for drug discovery. The aims of

structural genomics are to (i) explore the naturally occurring "protein fold space" and (ii) contribute to the characterization of function through the assignment of atomic resolution three-dimensional (3D) structures to proteins. It is now generally acknowledged that NMR will play an important role in structural genomics. The resulting demand for HTP structure determination requires fast and automated NMR data collection and analysis protocols.

The establishment of a HTP NMR structural genomics pipeline requires two key objectives in data collection. Firstly, the measurement time should be minimized in order to (i) lower the cost per structure and (ii) relax the constraint that NMR samples need to be stable over a long period of measurement time. The recent introduction of commercial cryogenic probes promises to reduce measurement times by about a factor of ten or more, and will greatly impact the realization of this first objective. Secondly, reliable automated spectral analysis requires recording of a "redundant" set of multidimensional NMR experiments each affording good resolution (which requires appropriately long maximal evolution times in all indirect dimensions). Concomitantly, it is desirable to keep the total number of NMR spectra small in order to minimize "interspectral" variations of chemical shift measurements, which may impede automated spectral analysis. Straightforward consideration of this second objective would suggest increasing the dimensionality of the spectra, preferably by implementing a suite of four- or even higher-dimensional NMR experiments. Importantly, however, the joint realization of the first and second objectives is tightly limited by the rather large lower bounds of higher-dimensional TR NMR measurement times if appropriately long maximal evolution times are chosen.

Hence, "sampling limited" and "sensitivity limited" data collection regimes are distinguished, depending on whether the sampling of the indirect dimensions or the sensitivity of the multidimensional NMR experiments "per se" determines the minimally achievable measurement time. As a matter of fact, the ever increasing performance of NMR spectrometers will soon lead to the situation where, for many protein samples, the sensitivity of the NMR spectrometers do not constitute the prime bottleneck determining minimal measurement times. Instead, the minimal measurement times encountered for recording conventional higher-dimensional NMR schemes will be "sampling limited," particularly as high sensitivity cryoprobes become generally available. As structure determinations of proteins rely on nearly complete assignment of chemical shifts, which are obtained using multidimensional ^{13}C, ^{15}N, ^{1}H- TR NMR experiments, the development of TR NMR

techniques that avoid the sampling limited regime represents a key challenge for future biomolecular NMR methods development.

Reduced dimensionality (RD) TR NMR experiments, designed for simultaneous frequency labeling of two spin types in a single indirect dimension, offer a viable strategy to circumvent recording NMR spectra in a sampling limited fashion. RD NMR is based on a projection technique for reducing the spectral dimensionality of TR experiments: the chemical shifts of the projected dimension give rise to a cosine-modulation of the transfer amplitude, yielding in-phase peak pairs encoding n chemical shifts in a n-1 dimensional spectrum. As a key result, this allows recording projected four-dimensional (4D) NMR experiments with maximal evolution times typically achieved in the corresponding conventional 3D NMR experiments. Furthermore, axial coherences, arising from either incomplete insensitive nuclei enhanced by polarization transfer (INEPT) or heteronuclear magnetization, can be observed as peaks located at the center of the in-phase peak pairs. This allows both the unambiguous assignment of multiple doublets with degenerate chemical shifts in the other dimensions and the identification of peak pairs by symmetrization of spectral strips about the position of the central peak. Hence, observation of central peaks not only restores the dispersion of the parent, higher-dimensional experiment, but also provides access to reservoir of axial peak magnetization. Historically, RD NMR experiments were first designed to simultaneously recruit both ^{1}H and heteronuclear magnetization for signal detection, a feature that has also gained interest for improving transverse relaxation-optimized spectroscopy (TROSY) pulse schemes. Moreover, RD two-spin coherence NMR spectroscopy subsequently also called zero-quantum/double-quantum (ZQ/DQ) NMR spectroscopy, served as a valuable radio-frequency (r.f.) pulse module for measurement of scalar coupling constants and cross-correlated heteronuclear relaxation.

Previously, a suite of RD $^{13}C,^{15}N,^{1}H$-triple resonance NMR experiments was presented for rapid and complete protein resonance assignment. Even when using short measurement times, these experiments allow one to retain the high spectral resolution required for efficient automated analysis. "Sampling limited" and "sensitivity limited" data collection regimes were defined, respectively, depending on whether the sampling of the indirect dimensions or the sensitivity of a multidimensional NMR experiments per se determines the minimally required measurement time. It was shown that RD NMR

spectroscopy is a powerful approach to avoid the "sampling limited regime", i.e., a standard set of experiments allows one to effectively adapt minimal measurement times to sensitivity requirements. It would be advantageous to implement those RD NMR experiments in a manner that allows frequency discrimination of the projected chemical shifts.

The present invention is directed to achieving these objectives.

The rejection of claims 94-133 under 35 U.S.C. § 101 for statutory type double patenting over claims 1-40 of U.S. Patent No. 7,141,432 to Szyperski ("Szyperski") is respectfully traversed.

It is the position of the U.S. Patent and Trademark Office ("PTO") that claims 94-133 of the present application recite the "same invention" as claims 1-40 of Szyperski, because all pulse sequences recited in Szyperski are performed in a "phase sensitive manner" as recited in claims 94-133 of the present application. Applicants respectfully disagree.

Claims 1-40 of Szyperski are drawn to a method for obtaining rapid and complete assignments of chemical shift values of ^{1}H, ^{13}C and ^{15}N of a protein molecule comprising: providing a $^{15}N/^{13}C$-labeled protein sample; and conducting four RD NMR experiments on the protein sample, where (1) a first experiment is selected from the group consisting of a RD 3D $\underline{H}^{\alpha/\beta}\underline{C}^{\alpha/\beta}$(CO)NHN NMR experiment, a RD 3D HA,CA,(CO),N,HN NMR experiment, and a RD 3D H,C,(C-TOCSY-CO),N,HN NMR experiment for obtaining sequential correlations of chemical shift values; (2) a second experiment is selected from the group consisting of a RD 3D HNNCAHA NMR experiment, a RD 3D $\underline{H}^{\alpha/\beta}$,$\underline{C}^{\alpha/\beta}$,N,HN NMR experiment, and a RD 3D HNN<CO,CA> NMR experiment for obtaining intraresidue correlations of chemical shift values; (3) a third experiment is a RD 3D H,C,C,H-COSY NMR experiment for obtaining assignments of sidechain chemical shift values; and (4) a fourth experiment is a RD two-dimensional (2D) HB,CB,(CG,CD),HD NMR experiment for obtaining assignments of aromatic sidechain chemical shift values.

Thus, claims 1-40 of Szyperski are not directed to "[a] method for obtaining assignments of chemical shift values of ^{1}H, ^{13}C and ^{15}N of a protein molecule comprising: providing a protein sample; and conducting four reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of ^{1}H and ^{13}C which are encoded in peak pairs of an NMR spectrum are detected in a

phase sensitive manner and (1) a first experiment is selected from … (emphasis added)" as set forth in claims 94-133 of the present application.

Applicants submit that claims 94-133 of the present application do not recite the "same invention" as claims 1-40 of Szyperski. In determining "same invention" type double patenting, courts have asked, for each claim at issue, whether the claim in one patent or application could be literally infringed without literally infringing the claim in the other patent or application. See, e.g., *In re Hallman*, 655 F.2d 212, 216, 210 U.S.P.Q. 609, 612 (C.C.P.A. 1981); *In re Avery*, 518 F.2d 1228, 1232, 186 U.S.P.Q. 161, 164 (C.C.P.A. 1975); *In re Vogel*, 422 F.2d 438, 441, 164 U.S.P.Q. 619, 621 (C.C.P.A. 1975). The PTO applies a similar test. Thus, section 804 of the Manual of Patent Examining Procedure ("MPEP") states that "[i]n determining whether a statutory basis for a double patenting rejection exists, the question to be asked is: Is the same invention being claimed twice? 35 U.S.C. § 101 prevents two patents from issuing on the same invention. 'Same invention' means identical subject matter." Further, the same section in the MPEP also states that "[a] reliable test for double patenting under 35 U.S.C. § 101 is whether a claim in the application could be literally infringed without literally infringing a corresponding claim in the patent. *In re Vogel*, 422 F.2d 438, 164 U.S.P.Q. 619 (C.C.P.A. 1970). Is there an embodiment of the invention that falls within the scope of one claim, but not the other? If there is such an embodiment, then identical subject matter is not defined by both claims and statutory double patenting would not exist." Applicants submit that such an embodiment exists in the present case.

Thus, one embodiment of the invention of Szyperski relates to a method where the four RD NMR experiments are conducted on the protein sample under conditions effective to cosine modulate the ^{13}C chemical shift evolution with the chemical shift evolution of ^{1}H and where the NMR signals are processed to generate a 3D or 2D NMR spectrum with one or more peak pairs derived from the cosine modulating (see col. 3, line 59 – col. 7, line 33 of Szyperski). This embodiment, however, does not enable the detection of the chemical shift value of ^{1}H in a phase sensitive manner. Therefore, such an embodiment, while falling within the scope of claim 1 of Szyperski, clearly does not fall within the scope of claim 94 of the present application, which requires that "the chemical shift values of ^{1}H *and* ^{13}C which are encoded in peak pairs of an NMR spectrum are detected in a phase sensitive manner (emphasis added)".

Since there is an embodiment of the invention of Szyperski that falls within the scope of claim 1 of Szyperski, but not within the scope of claim 94 of the present application, applicants submit that claims 1-40 of Syperski and claims 94-133 of the present application do not define identical subject matter. Accordingly, the above statutory type double patenting rejection is improper and should be withdrawn.

Finally, applicants hereby request that the examiner consider the December 8, 2003, and December 15, 2004, Information Disclosure Statements, indicate such consideration by initialing the accompanying PTO/SB/08A forms, and return the initialed PTO/SB/08A forms with the next communication from the PTO.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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